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DOI:

[10.1016/j.bbi.2014.10.003](https://doi.org/10.1016/j.bbi.2014.10.003)

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Peer reviewed version

*Citation for published version (Harvard):*

Lacourt, TE, Houtveen, JH, Veldhuijzen van Zanten, J, Bosch, JA, Drayson, MT & Van Doornen, LJP 2015, 'Negative affectivity predicts decreased pain tolerance during low-grade inflammation in healthy women', *Brain, Behaviour, and Immunity*, vol. 44, pp. 32-36. <https://doi.org/10.1016/j.bbi.2014.10.003>

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## Short Communication

Negative affectivity predicts decreased pain tolerance during low-grade inflammation in healthy women

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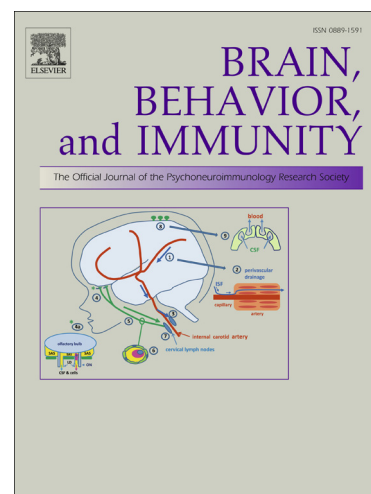
PII: S0889-1591(14)00476-0  
DOI: <http://dx.doi.org/10.1016/j.bbi.2014.10.003>  
Reference: YBRBI 2460

To appear in: *Brain, Behavior, and Immunity*

Received Date: 2 January 2014  
Revised Date: 2 October 2014  
Accepted Date: 3 October 2014

Please cite this article as: Lacourt, T.E., Houtveen, J.H., Veldhuijzen van Zanten, J.J.C.S., Bosch, J.A., Drayson, M.T., Van Doornen, L.J.P., Negative affectivity predicts decreased pain tolerance during low-grade inflammation in healthy women, *Brain, Behavior, and Immunity* (2014), doi: <http://dx.doi.org/10.1016/j.bbi.2014.10.003>

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**Negative affectivity predicts decreased pain tolerance during low-grade inflammation in healthy women**

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## Abstract

**Introduction:** Experimental animal studies provided evidence for a synergistic effect of immunological and psychological stressors on subsequent sickness behaviors. Up to now, little corroborating evidence for such synergy exists for humans, in whom it may provide a mechanism leading to the expression of functional somatic symptoms. The aim of the present study was to determine an interaction between stress(-vulnerability) and an immunological activation on experimental pain sensitivity, i.e., pressure pain threshold and tolerance in healthy humans.

**Methods:** In healthy female participants ( $n=25$ , mean age 22.3 years), negative affectivity (NA) and experienced stress were assessed by questionnaire before receiving a *Salmonella typhi* vaccine or saline control in a randomized blinded cross-over design. Pressure pain threshold was assessed at the lower back and calves and pain tolerance was assessed at the thumbnail, before and six hours after each injection.

**Results:** Vaccination induced leukocytosis (+100%) and increased serum IL-6 (+670%). NA predicted decreased pain tolerance after vaccination ( $\beta=-.57$ ,  $p=.007$ ), but not after placebo ( $\beta=.25$ ,  $p=.26$ ). Post-hoc analyses also demonstrated an association with administration order.

**Discussion:** NA moderated the effects of inflammation on pain tolerance. This finding is consistent with a synergistic model whereby inflammation may lower the threshold for pain reporting in individuals with increased vulnerability for somatic symptom reporting.

**Keywords:** inflammation; inflammatory response; interleukin-6; cytokines; pain sensitivity; algometry; pain tolerance; pain threshold; pressure pain; experimental pain; vaccine; placebo; randomized control; stress; life events; negative affectivity; negative affect; human;

## 1. Introduction

In reaction to an immunological challenge, immune-to-brain communication leads to a constellation of reversible behavioral and affective changes, denoted as sickness behavior (Hart, 1988; Kent et al., 1992). These include lethargy, social withdrawal, and enhanced sensitivity to pain (Larson and Dunn, 2001; Yirmiya et al., 1994). Animal studies have demonstrated that (psychological) stress factors may sensitize this immune-to-brain communication, resulting in a prolonged or more severe expression of sickness behavior symptoms when an immunological stressor is applied (Anisman et al., 2007; Gibb et al., 2013). Sensitization for sickness behaviors may thus entail dual involvement of immunological and psychological factors.

Such synergism might be applicable to humans as a possible explanatory mechanism for the experience of somatic symptoms or syndromes that cannot be fully explained by a present medical condition (i.e., functional somatic (FS) syndromes), such as in irritable bowel syndrome, fibromyalgia, and chronic fatigue syndrome (Dantzer, 2005). An interesting feature of such a dual-factor or interactive model is that it could link two independent observations: First, that FS symptoms are associated with a history of elevated stress exposures (Anderberg et al., 2000; Heim et al., 2006) and second, that FS symptom onset appears linked with immune activation or an increased infection load (Lacourt et al., 2013; Moss-Morris and Spence, 2006).

The aim of the current study was to investigate the possible synergistic effects of psychological stress(-vulnerability) factors and immunological activation on pain sensitivity. Increased pain sensitivity (i.e., a lower threshold for describing a stimulus as painful and a reduction in the level of pain that is considered tolerable) is a prominent symptom in many FS syndromes (Bradley, 2008; Stabell et al., 2013) and a typical aspect of sickness behavior with a reasonable degree of objectivity in humans (c.f., Benson et al., 2012). We assessed perceived

stress, cumulative life events, and negative affectivity (NA) as psychological vulnerability factors in healthy subjects, followed by a (placebo-controlled) *Salmonella typhi* vaccine.

Specifically, we tested the hypothesis that psychological vulnerability factors and immune activation by vaccination jointly enhance pain sensitivity. The present study was limited to women as they show a substantially higher prevalence of FS symptoms (Fink et al., 2007).

## 2. Methods

### 2.1 Participants

The present study involved 27 healthy non-smoking female participants, recruited among students and staff of the University of Birmingham by poster advertisement and word of mouth.

Inclusion criteria were an age between 18 and 50 years old and use of oral contraception.

Excluded were volunteers reporting diabetes mellitus, asthma, congestive heart failure, any psychiatric disorder, recent history of cancer, inflammatory disease, cardiovascular disease, chronic obstructive pulmonary disorder, wearing a pacemaker, and those taking any medications with known immune modulatory properties. Finally, participants were excluded if they were unwell on the day of testing or fell ill between the two test-days. Eligible persons received either a monetary incentive or credits for participation. The study protocol was approved by the Health Research Authority NRES Committee West Midlands – South Birmingham and all participants provided informed consent.

### 2.2 Study design

*Salmonella typhi* capsular polysaccharide vaccine (0.025 mg in 0.5 ml, Typhim Vi, Sanofi Pasteur, UK) was used as the immunological challenge. In a placebo-controlled cross-over

design, participants were tested in two conditions (i.e. vaccination or saline) on two test-days, separated by at least seven days. The order of conditions was counterbalanced across the participants and participants were blinded to the conditions.

### 2.3 Protocol

Informed consent was obtained on an initial appointment, which involved screening for inclusion and exclusion criteria and administration of questionnaires (see below). To reduce variance in anti-conceptive hormones within and across participants, participants with monophasic pills were tested during the three weeks of pill use, and participants with either biphasic or triphasic pills were tested within the first ten days after a stop week.

With the exception of the injection (vaccine or saline), both test-day protocols were identical. Participants reported between 8.00 and 11.00 a.m. at the Behavioral Immunology Laboratory of University of Birmingham, upon which baseline measures were obtained of heart rate, blood pressure, body temperature, and pain sensitivity. A blood sample was obtained approximately 15 minutes after arrival. Next, the participants performed several cognitive tasks (data not reported here), and were subsequently injected with either vaccine or saline followed by a 30 minute rest. All procedures were repeated at six hours post-injection, which coincides with a peak increase in vaccine-induced IL-6 and leucocytosis (Paine et al., 2013). Participants were allowed to leave the laboratory in between test-sessions.

### 2.4 Immunological measures

Blood samples were obtained by venipuncture and collected in vacutainers (Becton & Dickenson), and EDTA plasma and serum was subsequently extracted. Analyses for white blood

cell (WBC) count and differential were done using a Coulter counter (Beckman Coulter, Inc.).

Serum was analysed for the inflammatory markers interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ) using high sensitivity ELISAs (Quantikine HS Human IL-6 ELISA and Quantikine HS Human TNF- $\alpha$ , both R&D Systems, UK) in accordance with the manufacturer's instructions. The reported sensitivity of the assays was 0.039 pg/ml and 0.106 pg/ml for IL-6 and TNF-  $\alpha$  respectively, with recorded intra-assay and inter-assay variations both <10%.

## 2.5 Stress sensitivity and psychological stress

As a measure of stress sensitivity, negative affectivity (NA) was measured with the NA subscale of the Positive and Negative Affect Scale (PANAS) (Watson et al., 1988). Psychological stress was measured with the Perceived Stress Questionnaire (PSQ) (Levenstein et al., 1993) and the Life Events Scale for Students (LESS) (Clements and Turpin, 1996; Linden, 1984). A detailed description of all scales is provided in the online supplement.

## 2.6 Pain sensitivity

Pressure pain threshold and pressure pain tolerance were assessed using a digital pressure algometer (FPX50; Wagner Pain Test<sup>TM</sup>, Greenwich, USA). The procedure for assessing pressure pain sensitivity has been described elsewhere (Lacourt et al., 2012). Briefly, a pressure was applied and gradually increased at a rate of 100 kPa/s until the participant indicated the force to become unpleasant (for pain threshold) or unbearable (for pain tolerance), upon which the pressure was released. Pain thresholds were assessed three times at four body points to obtain a general body measure: bilaterally at the calf muscle belly and on the lower back at the paraspinal muscles of L3. A mean pain threshold was calculated by averaging over the last two



measurements of all body points. Pressure pain tolerance was measured on the thumbnail of the non-dominant hand. Although a general body measure taken at multiple anatomical sites would have been preferable for this measure too, multiple measures of pain tolerance were considered too burdensome for the participants. Pain tolerance measurements were repeated (with a minimum of 30 s in between measurements) until the participant indicated that the pain tolerance level was reached. The highest obtained pressure was used as pain tolerance in the analyses.

These algometric pain sensitivity measures showed good test-retest reliability for tests repeated within one session (intraclass correlation coefficient (ICC) > .80) (Lacourt et al., 2012; Potter et al., 2006) and over days (ICC > .70) (Potter et al., 2006).

## 2.7 Statistical analyses

The questionnaires did not have any missing data (with the exception of the LESS item “parent losing job”, N=6) and normality was adequate for all scales without visual outliers. IL-6 and TNF- $\alpha$  plasma concentrations were log-transformed to gain a normal distribution, after which no visual outliers were detected. Pain threshold measures showed moderate skewness and were square root transformed. Transformed and non-transformed pain data yielded similar results and the latter were reported.

The effects of vaccination were analysed with 2 Condition (saline, vaccination) x 2 Time (pre-injection, post-injection) repeated measures analyses of variance (ANOVAs). The effects of NA and stress were analysed by separately adding these factors as covariates to the models (ANCOVAs). In case of a significant three-way interaction (Condition x Time x Stress), difference-scores were calculated for pain sensitivity and threshold ( $\Delta$  = post-injection scores minus pre-injection scores), resulting in  $\Delta$ -saline and  $\Delta$ -vaccine change-scores. Separate

regression analyses were then performed with the  $\Delta$ -variables as dependent variable and the psychological factor of interest as predictor. Bonferroni correction was used to account for multiple testing, resulting in an alpha of .016 for the ANCOVAs and .025 for the regression analyses. The manufacturer delivered vaccines from two lots, therefore, batch was included as additional factor in all analyses. Data were analysed using SPSS for windows 20.1 (IBM SPSS Inc, Chicago).

### 3. Results

#### 3.1 Sample characteristics

Two participants were excluded from the analyses because of illness between test-days. Mean age of the final sample ( $N = 25$ ) was 22.28 years ( $SD: 3.39$ , range: 18 – 30). Mean BMI was 22.90 ( $SD: 2.76$ , range: 18.93 – 29.74), six participants were overweight with a  $BMI > 25$ . Mean scores ( $SD$ , range) for NA were 19.40 (5.92, 10 – 33), for Perceived Stress 71.80 (12.93, 52 – 94), and for Life Events 283.56 (125.62, 73 – 495).

#### 3.2 Main effects of vaccination

Descriptives for the pain and immune outcomes are provided in Table 1. With the alpha set at .016 vaccination was found to significantly increase WBC (+100%; condition x time:  $F(1,23) = 42.19$ ,  $p < .001$ ) and IL-6 (+670%; condition x time:  $F(1,23) = 33.37$ ,  $p < .001$ ). No effect of vaccination was found for TNF- $\alpha$  (condition x time:  $F(1,23) = 0.14$ ,  $p = .71$ ). Batch did not show an interaction with condition x time in any of the analyses.

Vaccination did not exhibit an effect on pain threshold (condition x time  $F(1,23) = 0.24$ ,  $p = .63$ ) or pain tolerance (condition x time:  $F(1,23) = 1.23$ ,  $p = .28$ ). Post-hoc analyses showed a

main effect for condition order, indicating an association between vaccination and pain tolerance in those receiving the vaccine first (online supplement, Table S1).

### 3.3 Vaccine-by-stress interaction effects

A significant 3-way interaction was found for NA with pain tolerance as dependent variable (condition x time x NA:  $F(1,22) = 9.45, p = .006$ ). Subsequent regression analyses (alpha set at .025) showed that NA was related to a decrease in pain tolerance (i.e., a negative  $\Delta$ -score) in the vaccine condition ( $\beta = -.57, t(22) = -2.96, p = .007$ ) but not in the saline condition ( $\beta = .25, t(22) = 1.16, p = .26$ ) (see Figure 1). Post-hoc analyses provided some indication that this relation between NA and vaccination on  $\Delta$ -pain tolerance was apparent for participants that received vaccination on their first test-day ( $r_{\text{spearman}} = -.76, p = .01$ ) and not for participants that received vaccination on their second test-day ( $r_{\text{spearman}} = .13, p = .64$ ). No significant interactions were found for NA with pain threshold as dependent variable or for the Perceived stress and Life Event measures.

## 4. Discussion

The current study set out to test the hypothesis that inflammatory mechanisms and stress or stress-vulnerability may interactively determine pain sensitivity. In support of this idea, the results showed that higher levels of NA were associated with lower tolerance to pain during vaccine-induced low-grade inflammation. To our knowledge, our results are the first experimental evidence from human studies that NA and inflammatory factors can interact to induce increased expression of pain symptoms, reflecting that NA may induce sensitivity for this aspect of sickness behaviour in humans. NA is one of the psychological factors most robustly

linked to physical symptom reporting and an established vulnerability factor for the experience of FS symptoms (Smolderen et al., 2007; Van Diest et al., 2005; Watson & Pennebaker, 1989). While both high NA and immunological triggers are associated with the development of functional somatic symptoms (e.g., De Gucht, 2002; Lacourt et al., 2013), neither of these factors alone seems to have sufficient predictive value. The interaction between NA and inflammation reported here is proposed as an explanation for this observation, proposing that both factors might be required. The here reported interaction between NA and inflammation is proposed as an explanation for this observation by implying that both factors are needed.

NA, but not perceived stress or life events interacted with inflammation to increase pain sensitivity. NA reflects proneness to subjective distress and unpleasurable engagement (Watson et al., 1988), and is highly correlated with the personality trait neuroticism (Wilson and Gullone, 1999). Perceived stress and life events on the other hand represent the experience of stress. Possibly, it is the more general negative state as opposed to specific events or experiences that primes the system for inflammatory effects on pain sensitivity.

The effects of systemic inflammation on pain sensitivity and the moderating effect of psychological stressors have been well described in animal models (Anisman et al., 2007; Gibb et al., 2013). These models identified as key mechanism the activation of glia cells by inflammatory mediators, which in turn alter neuronal functioning along the pain pathway (Hains et al., 2010; Watkins et al., 2007). Recently a mechanism was proposed by Frank et al. (2013) to explain how stress acts on central immune regulation, based on data showing that stress-related increases of glucocorticoids (which typically have an anti-inflammatory effect) can also enhance inflammatory responses to stressors, probably by sensitization of glia cells (Frank et al., 2013). Accordingly, high NA individuals might (as a result of frequent perceived stress) have primed

microglia cells for new challenges. These findings from experimental animal studies provide material for further mechanistic studies in humans.

NA predicted vaccine-induced changes in pain tolerance but not pain threshold. Although pain threshold and pain tolerance are both considered measures of pain sensitivity, there is evidence that they represent different processes: An earlier study using the same pain assessment methodology showed that pain tolerance is only modestly correlated to pain threshold ( $r \approx .40$ ) but moderately related to subjective sensory and affective pain ratings ( $r \approx .60$ ) (Lacourt et al., 2012). Pain threshold on the other hand showed low correlations with pain ratings ( $r \approx .30$ ). Thus, pain tolerance seems to incorporate an affective aspect of pain sensitivity, while pain threshold seems to represent a more objective sensory aspect. The present data is suggestive of the idea that the interaction between NA and inflammation is related to emotional aspects of pain sensitivity. It has been proposed that sickness behaviour is a motivational state “that reorganizes perception and action” (Dantzer, 2001). In this view, the affective or emotional aspects of pain sensitivity would be expected to change during inflammation, as these aspects underlie the individual’s withdrawing behaviour that facilitate recuperation from infection. This affective aspect in pain sensitivity is also seen in FS syndrome patients, where relations between psychological distress and pain sensitivity measures are commonly reported (Bradley, 2008; Verne et al., 2001).

#### *4.1 Limitations and recommendations*

A number of limitations should be noted. First, state negative affect was not assessed in this study and it cannot be ruled out that the observed decrease in pain tolerance was a consequence of increases in negative mood state induced by low-grade inflammation. The possibility of such an effect of mood is tentatively suggested by the observation that the effect of NA and

vaccination on pain tolerance might depend on the order in which vaccination was administered (i.e., receiving the vaccine first), which might reflect a result of anticipatory anxiety. An effect of state-anxiety has recently been observed in an elegant study using the (more potent) endotoxin paradigm (Wegner et al., 2014). Thus, the notion of an effect of state mood deserves further exploration in future studies. Second, the sample used in this study was relatively small, leading to low power to detect smaller effects. Replication in better powered studies is needed to determine if the observed interactive effect for NA generalizes to other psychological vulnerability factors. Third, only healthy female participants were included which reduces generalizability of our results. Since gender differences have been reported for pain sensitivity (Fillingim et al., 2009), it is possible that such differences may also exist in inflammation-induced changes in pain sensitivity. Finally, two different batches of the *Salmonella typhi* vaccine were used, thereby possibly enhancing between-participants variance in immunological activity. However, batch was included as a factor in all analyses, and no batch effects could be demonstrated.

In summary, NA, a trait related to a negative or stressful interpretation of events and an established vulnerability factor for the development of FS symptoms, was found to interact with vaccine-induced low-grade inflammation on pain tolerance. These results are in line with animal models on the synergistic effects of inflammation and psychological stress on subsequent sickness behaviors and may open new approaches to understanding somatic symptom reporting in humans.

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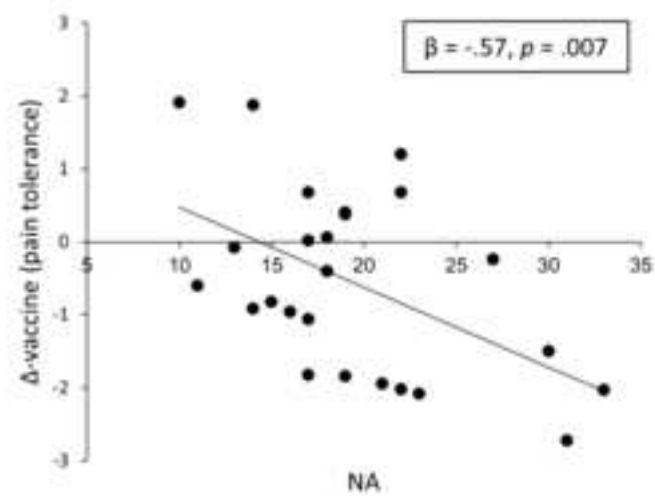
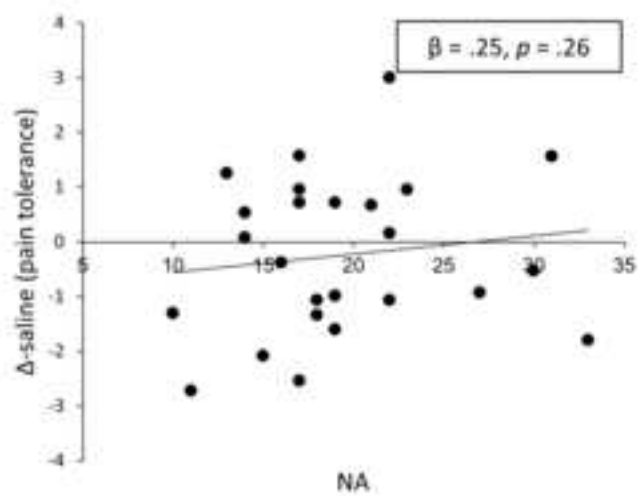
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Table 1. *Mean (untransformed)  $\pm$ SD of the dependent variables per assessment.*

Figure 1. *Scatterplot depicting the relation between NA and change in pain tolerance (a negative score indicates a decrease in pain tolerance from pre- to post-injection) within the saline condition (left panel) and the vaccine condition (right panel).*

	Saline		Vaccination	
	Pre-injection	Post-injection	Pre-injection	Post-injection
WBC ( $10^9/L$ )	$5.82 \pm 1.78$	$7.21 \pm 1.96^*$	$5.80 \pm 1.33$	$10.57 \pm 3.37^*$
Interleukin-6 (pg/ml)	$1.77 \pm 2.86$	$1.69 \pm 2.34$	$0.84 \pm 1.25$	$5.66 \pm 3.33^*$
TNF- $\alpha$ (pg/ml)	$2.00 \pm 3.37$	$5.37 \pm 9.35$	$3.89 \pm 6.17$	$6.45 \pm 9.99$
Pain threshold (kPa)	$391.27 \pm 186.55$	$405.02 \pm 175.53$	$398.15 \pm 150.26$	$392.27 \pm 148.79$
Pain tolerance (kPa)	$930.65 \pm 189.97$	$907.12 \pm 204.79$	$954.19 \pm 168.39$	$899.27 \pm 213.63$

\* Significantly different from Pre-injection at  $p < .001$ .



Stress sensitivity and immune activation interacted to increase sensitivity to pain.

Trait negative affect predicted lower pain tolerance under induced mild inflammation.

This interaction suggests a mechanism that may underlie functional pain symptoms.